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Arthrobotrys scaphoides from China and Europe with a phylogenetic analysis including the type strain

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Abstract —*Arthrobotrys scaphoides* is recorded for the first time from China. The strain is compared with European strains (including the type strain) morphological and molecular methods. The species is illustrated by photographs and line drawings. ITS sequence phylogenetic analysis places three *A. scaphoides* strains in the same monophyletic group with *A. conoides*, a species strongly different in morphology.

Key words —predacious fungi, rDNA gene

Introduction

Arthrobotrys Corda was first established for the type species, *A. superba* (Corda 1839), which is characterized by the formation of two-celled conidia on denticles in a whorled arrangement at the tip and the nodes of the simple, erect, septate conidiophore. The genus was long used in Corda's sense for nematode trapping hyphomycetes independent of the type of trapping organ present. Schenck et al. (1977) expanded the genus to include species with aseptate and multicelled conidia. Scholler et al. (1999) subsequently limited *Arthrobotrys* to only those species with adhesive networks, a concept we follow here.

In a survey of nematode-trapping fungi in China, we isolated a species of predacious hyphomycetes with rather large conidia and three-dimensional adhesive networks. Simultaneously, a European culture representing obviously

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Table 1. Morphological comparison of three *Arthrobotrys scaphoides* isolates with other nematophagous species possessing ± fusiform conidia.

Species	Conidia			Reference
	Size	# of septa	Shape	
A. scaphoides (YMF1.01895)	36.6-79.3 × 11.0-17.5	1-6, mainly 2-3	elongate fusiform	This paper
A. scaphoides (CBS 226.52)	26-83 × 12-17	1-3	elongate fusiform	Peach 1952
A. scaphoides (H.B. 6972)	50-86 × 13-16	2-4, mainly 3	elongate fusiform	This paper
A. gampsospora (Drechsler) S. Schenck et al.	25-76 × 7-16	4	elongate fusiform	Liu & Zhang 1994
Dactylellina copepodii (G.L. Barron) M. Scholler et al.	56-97 × 8.5-16	(1-)4(-6)	fusiform	Barron 1990
"*Dactylella*" *dianchiensis* Y.E. Hao & K.Q. Zhang	37.5-100 × 10-17.5	1-7, mainly 2-5	elongate fusoid to fusoid-clavate	Hao et al. 2004
A. mangrovispora Swe et al.	25–50 × 12–24	0-3, mainly 2	top-shaped to fusiform	Swe et al. 2008
A. microscaphoides Xing Z. Liu & B.S. Lu 1993	23-39 × 8 -15.5	0-3, mainly 2	top-shaped	Liu & Lu 1993

the same species was identified as *A. scaphoides*, a rather rarely reported taxon.

Our primary aims of this study were (i) to examine morphological differences between the two *A. scaphoides* isolates, (ii) to explore the ITS rDNA sequence similarity between the two isolates and the type strain, and (iii) to investigate molecular relationships between *A. scaphoides* and other nematode-trapping fungi.

Materials and methods

Origin and isolation of strains, documentation

Soil samples from Gansu Province, China, were sprinkled on corn meal agar (CMA, 20 g corn meal, 18g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) plates inoculated with the free-living nematode *Panagrellus redivivus* Goodey at 25°C. In a collection of *Orbilia* aff. *auricolor* (A. Bloxam ex Berk. & Broome) Sacc., on *Scirpus maritimus* from the Netherlands (deposited in the private herbarium of H.O. Baral—H.B.), *A. scaphoides* grew as contaminant with *A. oudemansii* M. Scholler et al. in an ascospore isolate of the *Orbilia*. Conidia of *A. scaphoides* were placed on half-strength CMA (without antibioticum, Rubner 1996) to which unidentified nematodes were later added. After incubation of about one month, samples were examined under a dissecting microscope. Isolates were taken and cultivated on CMA at 28°C and 22°C, respectively.

Morphological characters were observed and photographed with an Olympus BX51 and a Zeiss Standard 20 microscope. Trapping organs were induced by adding about 100 nematodes into a 1 × 1 cm square slot at the margins of the colony where the agar was removed.

DNA extraction, PCR amplification, and sequencing

Three *A. scaphoides* strains (including the type strain) were sequenced in the present study. Genomic DNA in the Chinese strain was extracted from the mycelium collected from single-spore cultures growing on cellophane membrane on PDA according to Jeewon et al. (2002). Primer pairs ITS5 & ITS4 (White et al. 1990) were used to amplify the complete internal transcribed spacer (ITS, including 5.8S). The PCR amplification parameters were: 1 minute initial denaturation at 95°C, followed by 30 cycles of 1 minute denaturation at 94 °C, 1 minute primer annealing at 50°C, 1.5 minutes extension at 72°C, and a final 10 minute extension at 72°C. The purified PCR products were directly sequenced on both strands with the same primers that were used for amplification.

The two European strains (including the ex-type culture, CBS 226.52) were extracted and amplified according to Hagedorn & Scholler (1999), with the deviation of using primers NS7 to NL4 and purifying with QIAquick Purification Kits. The result was bi-directionally sequenced using Amersham Thermo-Sequenase IRD-labeled primers (NS7, ITS5, ITS1, ITS4, NL1, and NL4; White & al. 1990) on a LI-COR 4000L automated DNA sequencer.

Phylogenetic analysis

DNA sequences were aligned with additional sequences obtained from GenBank using ClustalX 1.83 (Thompson et al. 1997) and adjusted manually using BioEdit sequence alignment editor. Parsimony analysis was run in PAUP* version 4.0b10 (Swofford 2002), with the following settings: gaps treated as missing data, all characters equally weighted, using heuristic searches with TBR (tree-bisection-reconnection) as branch-swapping algorithm, initial 'MaxTrees' setting at 100; bootstrap values were generated using the settings 1000 replications. GenBank accession numbers can be found in Fig. 4. Sequence similarity analysis among our *Arthrobotrys scaphoides* strains and other phylogenetic related species was performed by DNAMAN software. A maximum-parsimony analysis was performed based on ITS region of *A. scaphoides* and related predacious fungi (Fig. 4), the ITS rDNA alignment has 29 taxa, 559 aligned nucleotides, all characters were given equal weight, *Neurospora crassa* was selected as outgroup.

Results

Taxonomy

Arthrobotrys scaphoides (Peach) S. Schenck, W. B. Kendr. & Pramer, Can. J. Bot. 55: 984 (1977) Fig. 1–3

≡ *Dactylaria scaphoides* Peach, Trans. Br. Mycol. Soc. 35: 19 (1952)

≡ *Woroninula scaphoides* (Peach) Mekht., Khishchnye nematofagovye Griby – Gifomitsety: 113 (1979)

≡ *Monacrosporium scaphoides* (Peach) Xing Z. Liu & K.Q. Zhang, Mycol. Res. 98: 865 (1994)

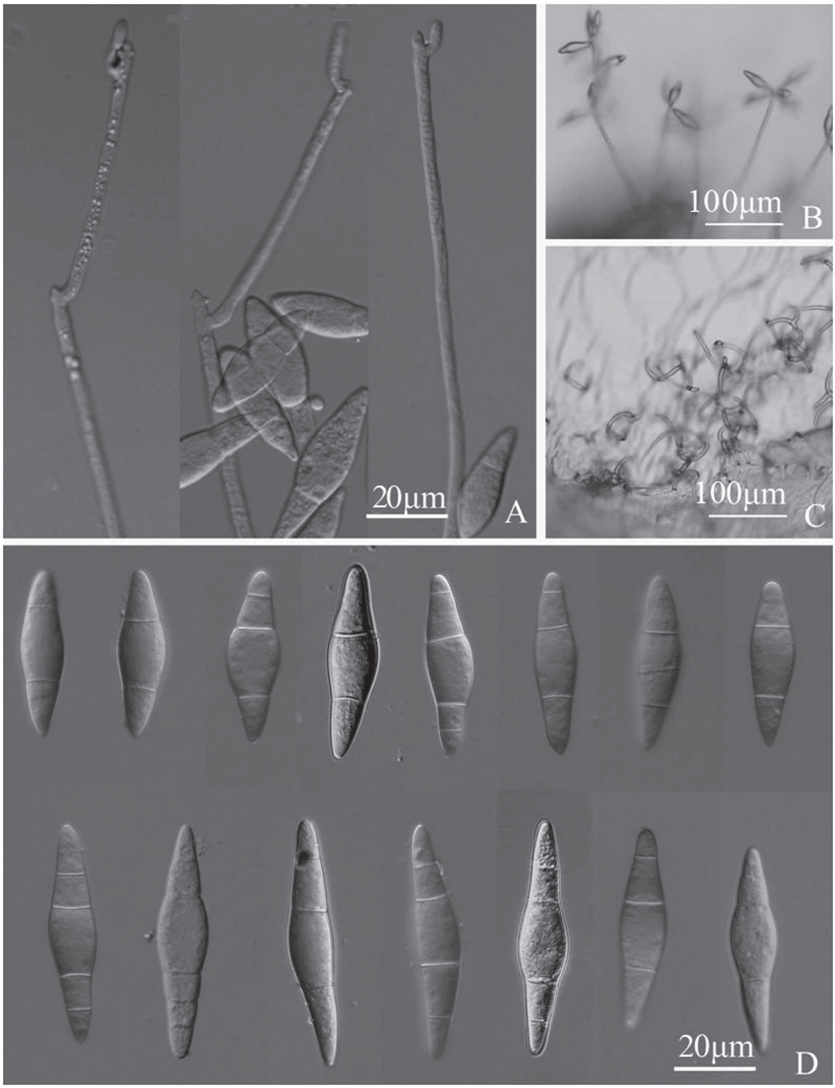

Fig. 1. *A. scaphoides* (YMF1.01895) A. Conidiophores. B. Conidiophores with conidia. C. Adhesive networks. D. Conidia (living state).

Origin of isolates: **PR China**, Gansu Province, Jiuquan city, alt. 2450 m, from soil, under *Malus asiatica* Nakai (*Rosaceae*), from a private plantation. VIII.2006, YMF 1.01895, permanent slide culture (YMF 1.01895); **The Netherlands**: Zeeland, old harbour facing the Hertoginpolder, Verdronken Land van Saeftinghe, most eastern part, alt. 0 m, on previous year's leaves of *Scirpus maritimus* L. (*Cyperaceae*), 23.III.2001,

G. Van Ryckegem (H.B. 6972b, dry specimen, associated with *Arthrobotrys oudemansii* M. Scholler et al. and its teleomorph).

Mycelium spreading, growing slowly, reaching 30 mm in diameter at 28°C after 12 days. Vegetative hyphae hyaline, septate, 4–6 µm wide. Conidiophores erect, hyaline and septate, unbranched, 4.5–5.6 µm wide at base and tapering to a width of 4–4.5 µm, at a distance of 80 to 200 µm from the base producing

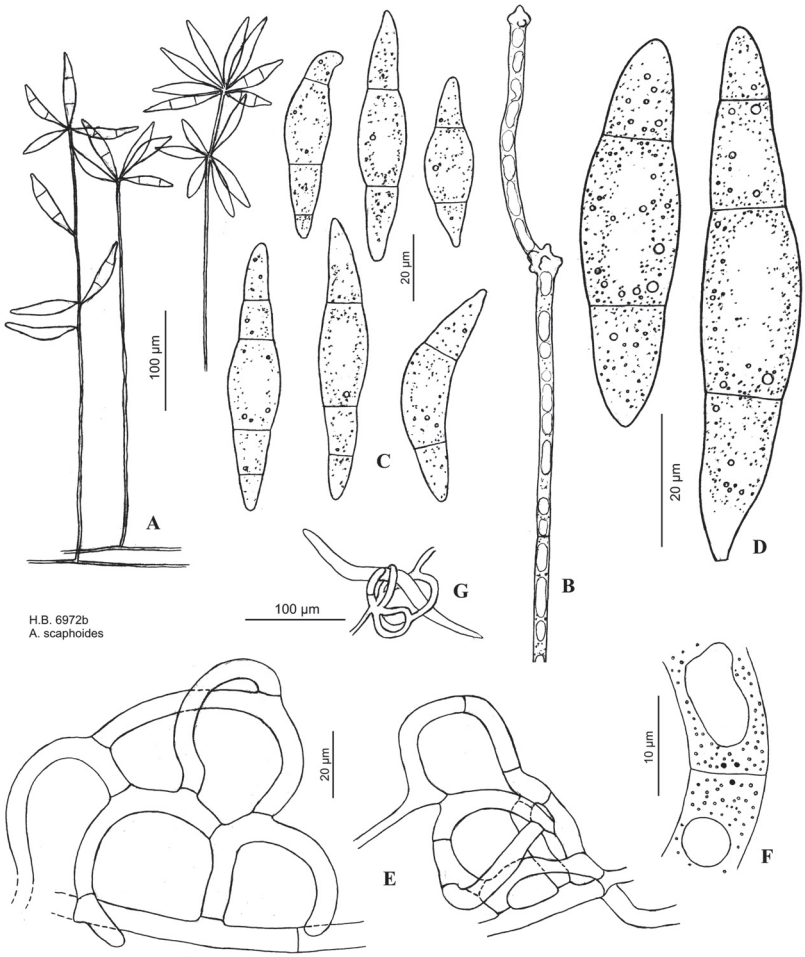

Fig. 2. *A. scaphoides* (H.B. 6972b) A. Conidiophores with conidia. B. Conidiophore. C–D. Conidia. E. Adhesive networks. F. Detail of adhesive networks showing vacuoles, three Woronin bodies close to septum, and many minute lipid bodies. G. Nematode trapped by adhesive network. All figures in living state.

1–6 conidia, occasionally up to 10 conidia, in a loose capitate arrangement, then following repeated elongation often giving successively rise to up to 3 additional conidial clusters by branching at or just below slightly swollen warted nodules, producing more or less geniculate conidiophores up to 365–430 µm long. Conidia hyaline, 36.6–79.3 (57.0) × 11.0–17.5 (14.0) µm (Chinese strain), (50–)60–80(–86) × (13–)14–15(–16) µm (Dutch strain, both vital state in water), fusiform, not or slightly curved, 1–6-septate, mainly 2–3 septate, the proportion of conidia with 1, 2, 3, 4, 5 and 6 septa was 1.3%, 48.8%, 37.5%, 10%, 1.3% and 1.3% respectively (Chinese strain), 2–3(–4)-septate (Dutch strain), middle cell mostly distinctly longer and wider than other cells. Three-dimensional adhesive networks observed when nematodes were added. Chlamydospores not observed in cultures.

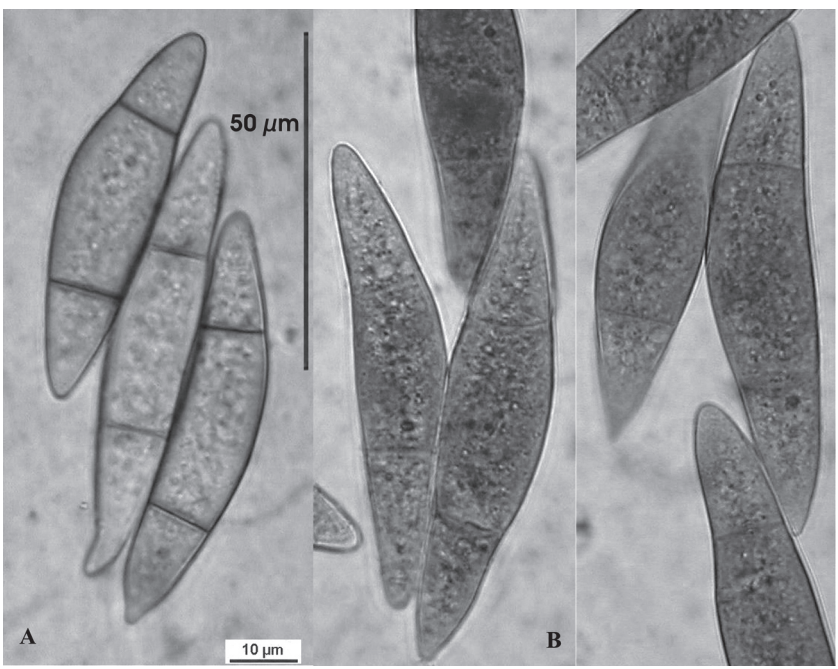

Fig. 3. *A. scaphoides* (H.B. 6972b) A–B. Conidia, vital state, stained by aqueous Cresyl blue (A) and Lugol's solution (B).

Phylogenetic analysis

We compared rDNA sequences from our fungus with related species bearing different predacious devices. The maximum parsimony analysis of the ITS rDNA sequences indicates that the investigated species grouped together

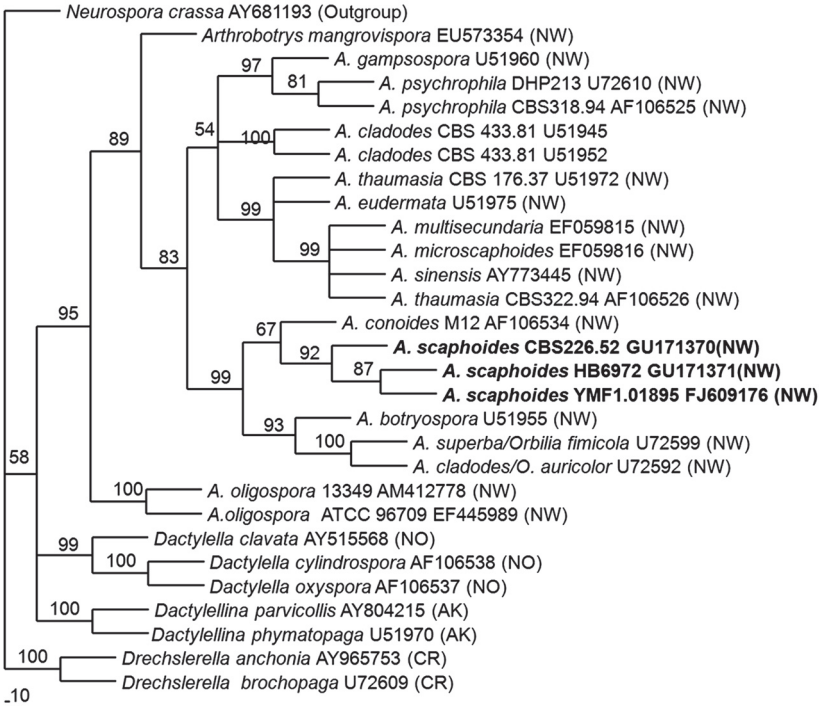

Fig. 4. Most parsimonious phylogenetic tree generated from a heuristic search based on the alignment of the ITS region sequences of predacious fungi. Numbers above lines represent bootstrap values from 1000 replicates on all parsimony-informative characters (only bootstrap >50% shown). The accession number of sequences obtained from GenBank are shown. CR = constricting rings, NW = networks, NO = no-trapping advice, AK= adhesive knobs. Tree length = 823, consistency index (CI) = 0.6452, homoplasy index (HI) = 0.3548, retention index (RI) = 0.7579, rescaled consistency index (RC) = 0.4890.

in accordance with the results of Scholler et al. (1999). The ingroup separated into four major clades based on unique types of trapping organs: one includes the species producing constricting rings (*Drechslerella*), one adhesive knobs (*Dactylellina*), one adhesive networks (*Arthrobotrys*), and the fourth clade includes species without known trap organs (*Dactylella*).

Within *Arthrobotrys* the three investigated *A. scaphoides* strains clustered together with 92% bootstrap support, forming a clade together with an *A. conoides* strain (bootstrap value 67%). This group forms with 99% bootstrap support a sister clade to the group including *A. superba*, *A. cladodes* var. *macroides* Drechsler, and *A. botryospora* G.L. Barron. Most remaining

Arthrobotrys species included in our analysis clustered in a clade sister to the two former groups.

Within *A. scaphoides*, the Chinese strain shows a closer relationship to strain H.B. 6972: the ITS rDNA sequence analysis demonstrates that there is 99.6% similarity between them (2 nucleotide variance in ITS1-5.8s-ITS2 region), but 98.7% between the Chinese strain and CBS 226.52 (type strain, 7 nucleotide variances), and 99.0% between the strain from *Scirpus* and the type strain from *Typha* (5 nucleotide variance). Between the Chinese strain of *A. scaphoides* and other morphologically close species, the similarity is 84.2% to *A. microscaphoides* Xing Z. Liu et B.S. Lu, 84.9% to *A. thaumasia* (Drechsler) S. Schenck et al., (CBS 322.94), and 85.7% to *A. gampsospora*.

Discussion

Arthrobotrys scaphoides was described by Peach (1952) as *Dactylaria scaphoides* from an aquatic habitat, with macroconidia 26–83 × 12–17 µm, (1–)2(–3) septate. Shorter conidiophores emerging from these conidia produced much smaller, non-septate microconidia (14–22 × 5–8.5 µm). Apart from the type collection (UK, England, Surrey, decaying leaves of *Typha latifolia* L., 1950, M.P. Peach, CBS 226.52), the rare reports of *A. scaphoides* include collections from Ireland (mixed deciduous leaf litter, 1982, Gray 1984), Africa (Morocco, 1200 m alt., from soil, 1993, A. Rubner, CBS 396.93), and S. America (Brazil, Minas Gerais, 426 m alt, from sheep faeces on a pasture, 1996, Saumell et al. 2000). Our Chinese specimen was isolated from the rhizosphere soil of a fruit tree (*Malus asiatica*) planted in arid areas of northwest China, while the Dutch strain was isolated from a monocotyledon substrate (*Scirpus*) in an environment comparable to the type strain. Ranges of conidium length and width in the Chinese strain are similar to those reported by Peach (1952) (Table 1), although the width range extended higher and the length range lower than those cited in the protologue. Variation in the Dutch strain was comparatively low, with conidial length at the upper limit. Microconidia were not observed in either of the two strains. Conidia with more than 4 septa were occasionally seen in the Chinese strain CMA culture but were absent in the two other strains, and 1-septate conidia were absent in the Dutch strain. The predacious ability using three-dimensional networks was found to be distinctly high in our fungus: the growth of networks and mycelium became particularly fast once the nematodes were added, and most nematodes were trapped by the network in only two days. Hao et al. (2005) found that nematode-trapping hyphomycete species common in terrestrial soil are also represented in aquatic environments. The same dimorphic habitat is found in the nematophagous fungus *A. scaphoides*, which has the capacity to occupy a broad range of habitats.

Among *Arthrobotrys* species with fusiform to top-shaped conidia, *A. gampsospora* most resembles *A. scaphoides*. It differs from the latter in the conidia being slightly narrower and partly more distinctly curved and in forming chlamydospores in pure culture. However, our phylogenetic analysis of an *A. gampsospora* strain from the type geographic region (Florida, U.S.A.) shows that it is not very closely related to *A. scaphoides.*

Both Liu & Lu (1993) and Swe et al. (2008) cite a strong similarity between *A. microscaphoides* and *A. mangrovispora* Swe et al. and *A. scaphoides*. However, *A. microscaphoides* has shorter, top-shaped conidia (also found in, e.g., *A. thaumasia*), and *A. mangrovispora* somewhat shorter and wider conidia of more variable shape (i.e., top-shaped but also elongate-fusoid as in *A. scaphoides*). Both differ by forming chlamydospores. Our ITS sequence analysis shows both species as phylogenetically distant to *A. scaphoides* (Fig. 4).

Dactylellina copepodii (G.L. Barron) M. Scholler et al., which also has conidia very similar to *A. scaphoides* in both size and shape, forms only one conidium at the conidiophore apex and captures copepods by adhesive knobs. The fusoid to fusoid-clavate conidia of "*Dactylella*" *dianchiensis* Y.E. Hao & K.Q. Zhang also resemble *A. scaphoides* conidia but differ in having up to 5(-7) septa and in lacking a larger central cell. Furthermore, its arcuate adhesive hyphal branches suggest this species more likely belongs to the genus *Dactylellina* (near the former *Gamsylella* species).

Classification based on conidial morphology is not supported by our phylogenetic tree, confirming the results of Scholler et al. (1999). For instance, the *A. scaphoides* clade, which contains *A. conoides* with much shorter, obovoid, 1-septate conidia, is closest to the *A. superba* clade where all members produce obovoid 0–1-septate conidia. On the other hand, the *A. scaphoides* clade is more remote to the clade containing *A. gampsospora*, *A. microscaphoides,* and *A. thaumasia* — with fusoid to top-shaped conidia with more than one septum — as well as species with cylindric-ellipsoid to obovoid, 1-septate conidia, like *A. cladodes*. This is astonishing because *A. gampsospora* conidia can easily be confused with those of *A. scaphoides*. Even more astonishing is the similarity of the *A. scaphoides* conidia to those of *Dactylellina copepodii,* which belongs in *Dactylellina* — as genetically demonstrated by Scholler et al. (1999).

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resource for microorganism strains in Yunnan Province.

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